# Utilization of genomic information in livestock improvement

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***Abstract:*** *Genomic information will increase selection efficiency in livestock. Genomics allows the tracing of transmission of genome fragments between generations, and the location and identification of genes whose polymorphisms partially explain quantitative trait variability. This information is useful for a better evaluation of the genetic values used by breeders, in particular when traits cannot be measured on a large scale for technical and/or economic reasons. It is also useful for reducing the generation interval through an early choice of breeding animals, and for increasing selection intensity. The first applications with regard to quality products and disease resistance are described in ruminant species. Interactions between genomic and reproductive biotechnologies are also described.*

***Keywords:*** *genomics; ruminant; selection; reproduction technologies*

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The artificial selection of animals for better productivity is a very old practice dating from the first domestication of major livestock species more than 10,000 years ago. As with other agricultural activities, animal breeding changed dramatically at the end of the nineteenth century with the modernization of agriculture. The development of quantitative genetics, statistical methods and computing sciences gave impetus to the genetic progress of animal populations. Selection schemes were organized on scientific bases, breeding stock were evaluated using increasingly sophisticated techniques, large-scale recording systems were implemented and artificial reproduction techniques multiplied the distribution of highly scored animals. The productivity of livestock increased by 1 to 2% per year due to the selection effort.

A second revolution in the breeding technologies started 25 years ago with the development of molecular biology. Major contributions were the discovery of restriction enzymes and the invention of the polymerase chain reaction (Saiki *et al*, 1985) allowing the use of numerous polymorphic genetic markers such as microsatellites and random amplified polymorphic DNA markers (RAPDs). The first genetic and cytogenetic maps of major livestock species were published in the 90s. Full sequencing of the bovine and porcine genome should be achieved within the next five years. It has been argued that this genomic revolution should further boost the genetic progress of selected populations. An abundance of literature has been produced exploring this opportunity, comparing strategies and evaluating their efficiency. Marker-assisted selection or introgression projects have been designed and implemented. Further progress should be obtained thanks to the identification of genes whose polymorphism is linked to a part of the phenotypic variability, explaining the quantitative trait loci (QTL) effects.

In this paper, the contribution of genomics to the genetic improvement of ruminants is explored. The elements for evaluating the efficiency of selection schemes are presented, and the way genomics could improve those elements are described.

## Basics of genetic progress

Quantitative geneticists have developed sophisticated modelling techniques to evaluate the way a selection plan or introgression programme might change the distribution of a trait in a population. Phenomena such as overlaps between generations, non-normal trait distributions, alternative hypotheses concerning the underlying genetic mechanisms (from the polygenic to the monogenic situation), limited size of the group of candidates, etc were considered when describing the evolution of the genetic mean and variability of the selected traits.

The 'archaic' but easily understandable modelling tells

us that the expected genetic progress $\Delta g$ is given by $\Delta g = i(q).\rho.\sigma/t$, ie is proportional to the selection intensity $i(q)$, the precision of the selection $\rho$, the available genetic variability $\sigma$, and inversely proportional to the generation interval $t$. The additive part of the genetic variability $\sigma$ is the major source of the progress, even if non-additive variance is exploited in crosses between breeds, and could be selected for in recurrent reciprocal selection strategies. A major concern is to maintain this genetic variability for as long as possible to allow further progress, for more and more complex breeding value evaluation methods and mating systems are being developed to meet this demand. A huge variety of genetic value estimations coexist depending on the species, the trait and the statistical technology. The precision of the evaluation, $\rho$, varies accordingly, and may be very high when the trait displays a high heritability coefficient, when the candidates are evaluated on a large number of data (large progeny groups), and when the statistical method used is able to exploit all available information. The selection rate $q$, the ratio of selected to candidate animals, depends on the reproduction parameters and the number of traits simultaneously selected.

## Contribution of molecular genetics to the improvement of animal breeding plans

From 1990 until now, the contribution of molecular genetics to livestock selection has essentially been through the possibility it gave for tracing the transmission of genome fragments from one generation to the next, and for identifying linkages between closely located fragments through their co-transmission. The most important application of those possibilities was the detection of QTL, and their selection. If a breeding animal is heterozygous at a marker locus (M1/M2) and at a linked QTL (Q1/Q2), most of its progeny receiving M1 (or M2) receive Q1 (or Q2). If the QTL polymorphism induces a large enough difference for a measured trait such as milk production or growth rate, the observation of a significant difference between groups of progeny having inherited M1 or M2 indicates the presence of the QTL. Marker-assisted selection is then possible in the next stage, keeping those progeny receiving the marker allele associated with the most favourable QTL allele, without any further phenotypic measurement.

Marker-assisted selection (MAS) has been extensively studied. From the founding work of Smith (1967) showing its possible efficiency, to the most recent papers such those of Gianola *et al* (2003) on marker-assisted prediction of genetic values, very different situations have been modelled and quite different estimates of the relative efficiency of MAS published (from more than 300% when compared with a classical phenotypic selection in Lande and Thompson, 1990, to negative figures in Ruane and Colleau, 1995). Contrary to plant life, in which homozygous lines are available allowing the use of global linkage disequilibria in their crosses, MAS in animals is presently exclusively based on within-family linkage disequilibria: association between marker and QTL alleles may vary between families (a sire may be M1Q1/M2Q2, while another will be M1Q2/M2Q1, and a third M1Q1/M2Q1). The major disadvantage of this situation is the necessity of evaluating the association for any new sire.

Nevertheless, MAS programmes have been organized in dairy cattle populations, in particular in New Zealand and France, where a project involving the industry (UNCEIA), research (INRA) and a national molecular laboratory (LABOGENA) has been implemented, with the aim of genotyping 10,000 individuals/year on at least 33 markers each (Boichard *et al*, 2002). In such programmes, the molecular genetics information gradually increases with the generations, as occurs with more classical phenotypic and genealogical information, allowing an automatic re-evaluation of the marker–QTL association and a refinement of their location.

Other applications were proposed that make use of this ability to trace transmission and co-transmission. For instance, genetic markers may help keep genetic variability in small populations (Chevalet, 1992) through the choice of breeding animals with a maximum proportion of heterozygous markers.

Even though the number of detected QTL is rapidly increasing in all major livestock species and many production traits, the genes responsible have been identified in only a few of them. It is indeed difficult to determine the precise location of a QTL for they generally have only a limited effect on phenotypic variance, and large efforts are needed to accumulate progeny data (utilization of the within-family linkage disequilibria) or to exploit ancestry information (utilization of the population linkage disequilibria). These difficulties should progressively vanish with the help of programmes aimed at producing bacterial artificial chromosome (BAC) libraries, full physical maps and, ultimately, complete genome sequencing, as well as information given by between-species comparisons, genetic maps and expressional genomics. Researchers have an increasing number of candidate genes for testing, either as possible genes corresponding to QTL already detected (if the candidate is located within the confidence interval of the QTL location), or in a reverse approach for cases in which the effect of their polymorphism on the economical trait is directly estimated.

In future, selection will not be based on markers linked to QTL, but directly on polymorphic genes controlling the trait's variability (known as direct gene selection, or DGS).

## Utilization of genomics for improving the precision of the selection ($\rho$)

Compared with classical schemes, the efficiency of MAS or of DGS will be maximal for traits whose genetic values cannot be precisely estimated. This is in cases when the number of offspring is limited in progeny-testing schemes due to the reproductive characteristics of the species (meat ruminants mainly bred by natural mating). This may also be the case if the heritability of the trait is limited due to difficulties in controlling environmental variability. A typical example is the seasonal reproduction of sheep, a trait very difficult to measure with precision in the field (neither hormonal nor ovulation rate measurements can be organized on farms, or there is no control of the mating period, thus making the use of the fertility hazardous as an indirect estimation of the start of the reproduction season). Pelletier *et al* (2000) showed that a

polymorphism in the melatonin receptor 1a gene (MTNR1A) was associated with seasonal reproduction in the Mérinos d'Arles breed, making possible a direct selection of rams on this trait (a selection experiment has been organized to validate this hypothesis: Bodin).

In many circumstances the traits are very costly to measure and selection cannot be organized on a large scale. The improvement of meat quality in cattle is limited by the difficulty due to the high cost of carcass destruction. In this species, meat tenderness is the most important criterion of quality to be improved. It could be estimated directly by a trained test panel or using indirect predictors such as Warner-Bratzler shear force. Both measurements display moderate heritability values, but neither can be organized on a large scale. The recent discovery of the role of polymorphism of the Calpain (Page *et al*, 2002) and Calpastatin genes (Nonneman *et al*, 1999) in tenderness variability opened a new, feasible approach to tenderness improvement. If this polymorphism exists in a given breed, and if its quantitative effect is confirmed in this breed, young bulls could be selected early on their genotype at these loci without any extra costly phenotypic estimation.

Intramuscular fatness is another trait of interest, linked to the juiciness and flavour. Indirect estimation using chemical assays, spectrophotometer or in vivo US measurements are currently being studied, but here again, it could be faster and easier to make use of genetic polymorphisms. Large QTL detection programmes have been organized in Australia and the USA. The thyroglobulin gene has been suggested as underlying one of these and its use patented (Renand).

The control of diseases is becoming a major concern in the livestock industry because it is linked to food safety, animal welfare and farm productivity. The exploitation of existing genetic resistance to disease is one of the powerful tools that should be developed in the future. However, for most diseases, the health status of animals is not, or is very poorly, recorded on farms and would not give a useful measurement of animals' ability to resist a given disease. Indeed, susceptibility is only assessed when the pathogen is present on the farm, a situation that is both undesirable and uncontrollable. The only solution is to organize a challenge on the candidates or on relatives under controlled conditions. This has been proposed for the resistance of sheep to internal parasites, measurable by artificial contamination of young male lambs when they have been gathered in breeding centres (Woolaston and Baker, 1996).

Recently developed selection programmes aimed at increasing the resistance of sheep to scrapie are a very convincing example of the usefulness of genomic approaches in improving these categories of traits. It is now well established that the polymorphism at the *PrP* gene is linked to the variability of resistance to transmissible spongiform encephalopathies in the human, mouse and ovine species. In sheep, one of the alleles (coding for alanine and arginine at codons 136 and 171 respectively) looks to be associated with a full resistance to natural scrapie and the absence of healthy carrier status (see Palhière *et al*, 2002, for a review). These results, observed in very different breeds and locations, form the basis of selection programmes at a national level in the UK, the Netherlands and France. Hundreds of thousands of blood samples are collected in the flocks and sent to laboratories, where DNA is extracted and *PrP* genotypes established using different techniques such as the TaqMan or the PCR-RFLP. The flow of genetic information is organized to allow the breeders to select their resistant animals as soon as possible. The success of these programmes comes from the agglomeration of a number of conditions making a genomic approach attractive: great difficulties in measuring the trait by classic means, the large effect of the gene on the selected trait, the absence of negative side effects, and probably most of all, the huge demand from consumers for control of the disease.

In practice, selection is always multipurpose and breeders have to face genetic antagonisms between traits, limiting the expected annual progress for each of them. For instance, selection for faster growth and a better muscle/fat ratio may lead to a deterioration in the flavour and colour of beef. A rather strong negative genetic correlation has now been established in sheep between direct and maternal effects on lamb growth. The role of genetic antagonism explained by pleiotropic opposite effects of genes could not be exploited easily, except by manipulating complementarities between breeds (for example, production of lambs from dams with a high genetic value for their mothering ability and terminal sires selected for growth rate). However, genomics will help resolve antagonisms in some situations. If the opposite effects came from two closely linked genes presenting unfavourable linkage disequilibria, the identification of the genes and a systematic research of recombinants in a large number of young candidates for selection should solve the problem. This was supposed, for instance, in the case of goats' polled intersexuality syndrome, associated with the absence of horns, a phenotype desired by breeders (Cribiu *et al*, 2000).

Another situation of interest is found in a population selected for a trait known to be under the control of a mixed inheritance with a major gene presenting deleterious side effects. This was the case in the Lacaune sheep population, a strain of which has been selected for prolificacy since the 1970s with such success that lamb mortality decreased dramatically. This fast improvement of litter size was recently explained by the segregation of a gene located on chromosome 11 (Lecerf *et al*, 2002). A new option for breeders of this strain is now to eliminate the carriers of the extreme allele at this locus and restart selection in a smoother way. Similar opportunities are currently considered in the Pietrain pig breed, which is characterized by an extreme body conformation, partly explained by their nn genotype at the Ryanodin receptor locus. This genotype is associated with an undesirable meat defect and stress susceptibility, and a few breeding companies are now willing to create NN Pietrain by a rapid introgression of the wild allele N.

## Utilization of genomics for increasing selection intensity $i(q)$

The success of the selection process is directly linked to the selection pressure that can be put on candidates differing by their estimated genetic value. When a large number of candidates is available, modern genomics may

not help to improve this. This is not the case, however, when selection is operated after a progeny test, a very costly and time-consuming procedure. In these situations, MAS offers a pre-selection of young bulls on marker information, before their progeny test. The extra gain predicted with this technique may reach 25%, as described by Kashi *et al* (1990) and Meuwissen and van Arendonk (1992).

If we assume that MAS and DGS will mainly be used for the selection of new traits such as disease resistance or meat quality, another very beneficial aspect is that the number of candidates will be largely increased because those new traits are generally not correlated, or are poorly correlated with the main selection objectives. Thus genomic-based selection will exploit cheap sources of improvement, presently devoted to the maintenance of breed standard traits or non-heritable characteristics.

A major change in selection practices and in the expected genetic progress will be obtained when techniques for the genomic selection of embryos become available. Embryo transfer is already largely used in dairy cattle selection schemes, and the increase in the number of ovocytes allowed by the Ovum Pick Up technology allows the production of large numbers of offspring/élite dams. It has been shown that biopsies done on embryos to genotype the extracted cells can be safe for the future calf (eg Nibart and Humblot, 1998). Research programmes are currently being organized in various countries (Belgium, France, New Zealand) to improve this technology. The final aim is the production of a large number of embryos/dam, their genotyping at a number of loci of interest after a biopsy of a few cells, the selection of the most interesting embryos via their genetic composition and their re-implantation in recipient females. A major effect of this protocol will be a large increase in the number of candidates for selection, at a relatively low cost (at least in the cattle species).

## Utilization of genomics for reducing the generation interval *t*

The multiple ovulation and embryo transfer (MOET) technique used in dairy cattle selection schemes is optimized for minimizing the generation interval. This is not the case in other ruminants such as beef cattle, in which the progeny test of the males, when organized, takes a long time before the results are obtained. Technological, financial or practical reasons often make the techniques used in MOET impossible: in sheep, the cost of the production of embryos is still too high and the technological results too variable for implementing this technology on a large scale. In these situations, MAS or DGS plans, in which young males would be selected on genomic information but not progeny tested, could be a way of reducing the generation interval. Such plans should be technically (and economically) evaluated, using, for instance, the modelling procedures described by Ruane and Colleau (1995).

Another contribution of genomic information in accelerating genetic progress is marker-assisted introgression, as described by Hospital *et al* (1992), Visscher *et al* (1996) and Hospital and Charcosset (1997). Introgression of a gene or a QTL from a donor to a recipient breed has already been organized in a few cases in ruminants. In France, the Booroola gene was, for instance, introgressed from Australian merino to Mérinos d'Arles and Romanov breeds (Mulsant and Elsen, 1995). The idea of Hospital and colleagues was to select, at each introgression generation, those animals that carried the higher proportion of marker alleles from the recipient breed. Animals from the second generation of the marker-assisted introgression process are, for instance, similar to the animals of the fourth generation of the classic introgression, hence the saving of two generations of introgression becomes a possibility. A project for introgressing the Texel gene in this way in recipient French breeds is currently being studied.

For the long term, Georges and Massey (1991) proposed a so-called 'velogenetics' procedure, mixing reproduction biotechnologies and modern molecular genetics. In their scheme, the generation interval is reduced by collecting oocytes from foetuses, maturing and fertilizing these oocytes in vitro, selecting the embryos on molecular information obtained after a biopsy and implanting the embryo in recipient females. More recently, Haley and Visscher (1998) proposed 'whizzogenetics', which completes the velogenetics by avoiding re-implantation of embryos in recipients, suggesting that meiosis could be induced, thereby producing gametes to be fertilized. Even if still unrealistic, these dreams of biotechnologists could become a reality in the next 20 years, with a dramatic potential change of the selection plans and of their efficiency, but with a high risk of rejection by society.

## Utilization of genomics for controlling the genetic variability σ

Genetic variability provides the 'energy' of genetic progress. Its control is essential if breeders want their selection schemes to be efficient in the long term. As indicated above, sophisticated mathematical tools have recently been developed to help this control. Molecular information can help improve this control, as Toro *et al* (1998, 1999) and Verrier and Rognon (2000) have described. On the other hand, marker-assisted selection of new traits, or the choice of breeding stock on their molecular index for the main selection objective operated within family, as proposed for most dairy cattle, should have only a very limited impact on existing variability.

From a more futuristic point of view, modern biotechnology could increase the available genetic variability. Transgenesis will probably become more precise, the cloning of somatic cells opening the perspective of homologous recombination. Fine manipulation of the genome will then be possible, for instance by accelerating introgression programmes (including, in a single step, a favourable allele in the genome of an élite bull) or adding alleles such as the sheep PrP resistant allele to cattle or goat genomes.

## Conclusions

Marker-assisted or direct gene selection are making use of genomic information, which is growing very rapidly with the organization of large-scale international programmes.

All aspects of selection plans are concerned with these new technologies. Globally, the genotyping of markers or genes will provide rapid and cheap information about many characteristics, including those traits that are difficult to measure and poorly heritable. However, molecular information by itself is not sufficient and will have to be coupled with measurements of the selected traits in finely controlled designs. As explained by Lande and Thompson (1990), selection practice will alternate between phases when the effects of genes are estimated and phases when selection on molecular information is possible.

Modern genetics cannot be dissociated from modern reproductive biotechnologies. MOET schemes are already running in dairy cattle. Further progress in the production of large numbers of embryos, their genotyping and selection is still needed before it becomes routine to make full use of genomics in breeding.

A number of useful QTL and a few genes have been detected in ruminants. Some of them are already used in practice. The *PrP* gene in sheep, and the organization of MAS in dairy cattle are two examples of the potential success of this new breeding.

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